STOCK Tg(tetO-SNCA*A53T)E2Cai/J

Stock No: 012442
Protocol 29807: Standard PCR Assay - Tg(tetO-SNCA*A53T)
Version 2.2

Notes

This assay will NOT distinquish hemizygous from homozygous transgenic animals .

This assay does not work well without the use of a Hotstart Taq polymerase.

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Expected Results

Transgene = 574bp
Internal positive control = 200bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
9936		TAC TGC TCC ATT TTG CGT GA		Transgene Forward	A	
9937		TCC AGA ATT CCT TCC TGT GG		Transgene Reverse	A	
oIMR8744		CAA ATG TTG CTT GTC TGG TG		Internal Positive Control Forward	A	
oIMR8745		GTC AGT CGA GTG CAC AGT TT		Internal Positive Control Reverse	A	

Reaction A

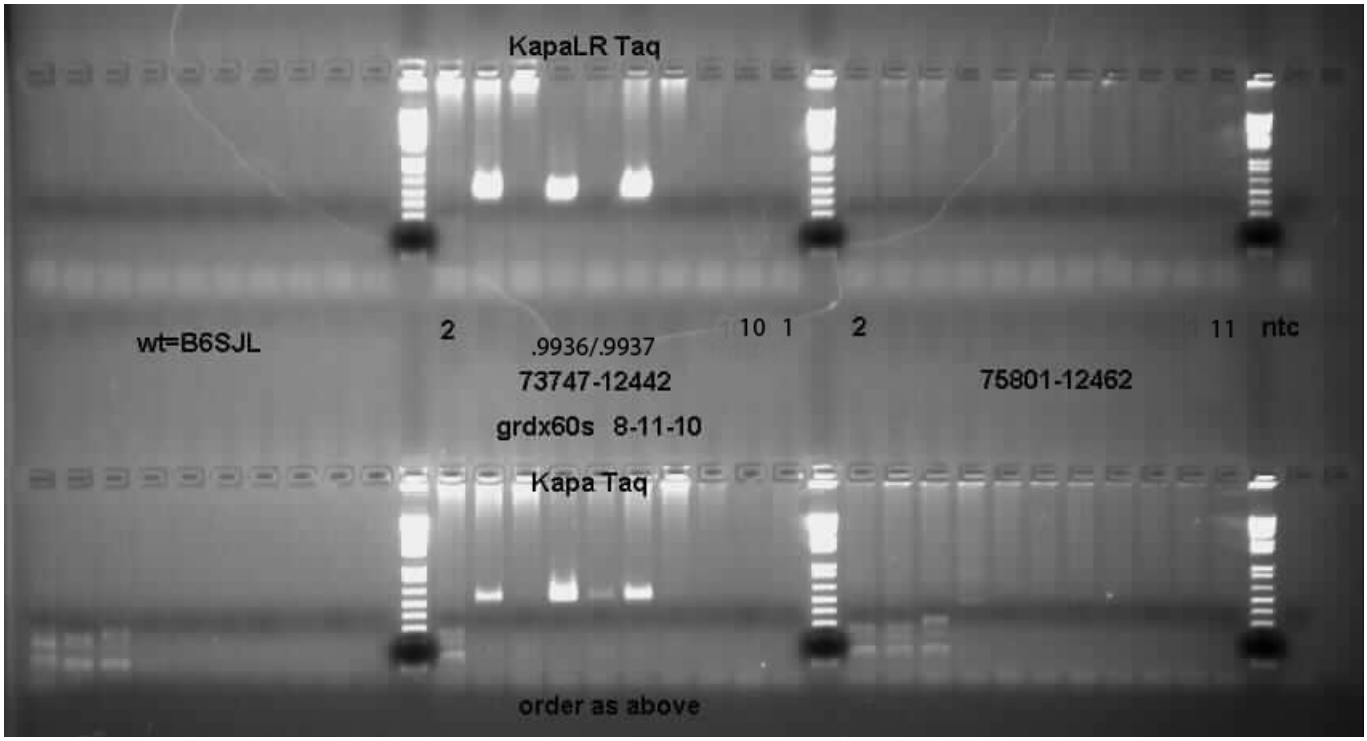
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
9936	0.50 uM
9937	0.50 uM
oIMR8744	0.50 uM
oIMR8745	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

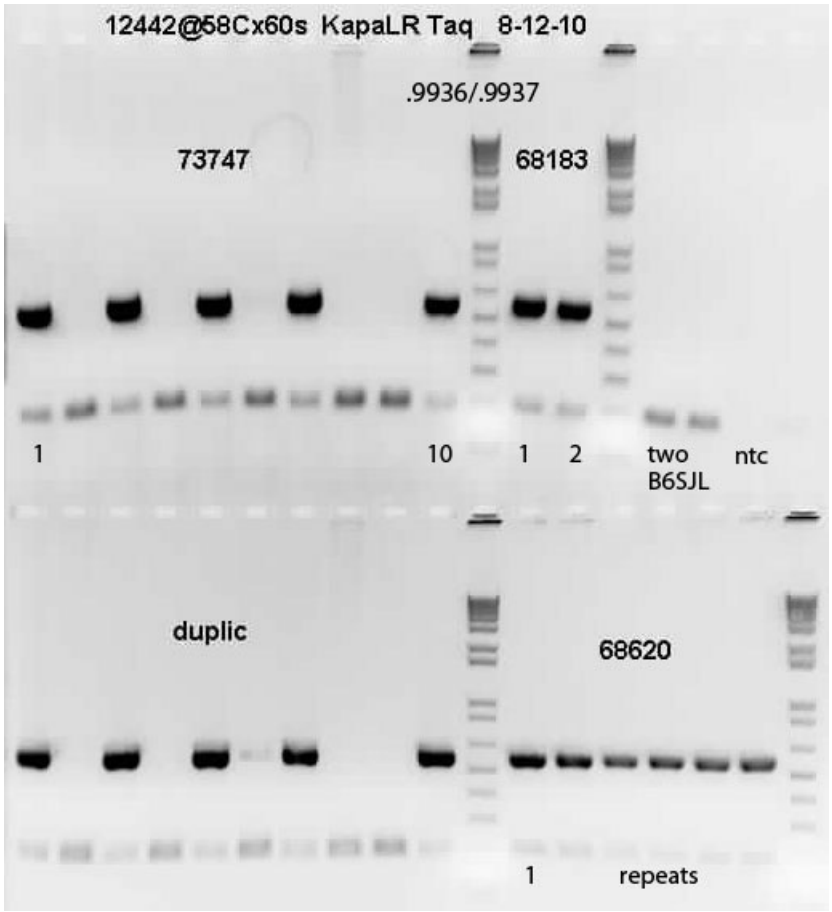
Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.


KapaLR Taq
wt=B6SJL
2
.9936/.9937
73747-12442
10 1
2
11 ntc
75801-12462
grdx60s 8-11-10
Kapa Taq
order as above


12442@58Cx60s KapaLR Taq 8-12-10
.9936/.9937
73747
68183
1
10
1
2
two B6SJL
ntc
duplic
68620
1
repeats